



1655

Patent  
Attorney's Docket No. 024705-083

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

HAYASHIZAKI

Application No.: 09/269,573

Filed: July 16, 1999

For: METHODS FOR DETECTING  
MUTATION IN BASE SEQUENCE

Group Art Unit: 1655

Examiner: B. Forman

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AUG 01 2001

TECH CENTER 1600/2900

COMMUNICATIONAssistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Further to the Reply & Amendment filed in connection with the referenced application on June 25, 2001 and in complete response to the Official Action mailed March 23, 2001, attached is the executed Declaration of Okazaki Yasushi.

In the event that there are any questions concerning this Declaration, or the application in general, the Examiner is respectfully urged to telephone the undersigned so that prosecution of this application may be expedited.

No fees are believed to be due by this paper. However, in the event that any fees are required, the Commissioner is hereby authorized to deduct such fees from Deposit Account No. 02-4800.

Respectfully submitted,

BURNS, DOANE, SWECKER &amp; MATHIS, L.L.P.

By:   
Malcolm K. McGowan, Ph.D.  
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(703) 836-6620

Date: July 30, 2001

(09/99)



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**DECLARATION OF OKAZAKI YASUSHI**

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

I, Okazaki Yasushi, hereby declare and state:

1. I am currently employed as Team Leader of the RIKEN Genome Science Laboratory, RIKEN Yokohama Institute, 1-7-22 Suehiro-cho Tsurumi-ku, Yokohama, Kanagawa, Japan. My Curriculum Vitae is attached to this Declaration.
2. I have reviewed the above-cited patent application, and the PCT publication WO 03/02216 of Wagner et al. (WO 93/02216), and the U.S. Patent Examiner's statements regarding this publication in the Official Action mailed March 23, 2001, in connection with the above-cited application.
3. It is my understanding that the Examiner is arguing that the use of a full-length gene as a hybridization partner is implicit in the disclosure of the PCT publication. I gained this understanding in part from the following passage from page 4 of the Official Action:  

Wagner is silent with regard to the fragment having all of a full-length gene. However, the sequence of a full-length gene recited in Claim 1 is deemed to be inherent in the DNA hybridization partner having a mRNA target in Wagner et al. because DNA hybridization partners of mRNA inherently encompass a full-length gene and therefore the DNA hybridization partners of Wagner et al. encompass the sequence of a full-length gene.

(09/99)

4. I must disagree with this conclusion. The Wagner et al PCT publication states first, at page 6, lines 26-27, that the hybridization *partner* is cDNA or a synthetic oligonucleotide. Then, at page 6, lines 27-28, the Wagner et al. PCT publication states that the hybridization *target* is mRNA. It is clear to me from these passages that Wagner et al are explicitly distinguishing between hybridization "partner" and "target". I conclude from these passages that the hybridization partner in the method disclosed in the Wagner et al PCT publication is a cDNA or oligonucleotide fragment, and not a full-length gene.

5. From my knowledge of hybridization technology, and my review of the Wagner et al. PCT publication, I understand the reference to cDNA in Wagner et al. to refer to EST sequences or shotgun fragments, and not to full-length genes. In fact, cDNA generally used in the scientific community are fragments of cDNA since full-length cDNAs are difficult to prepare and require specific protocols.

6. I believe that this is confirmed in the Wagner et al PCT publication at Example III, page 44, and Example IV, page 46, where the preparation of the cDNA molecule used as hybridization partner (not target) are prepared by "standards methods" (Sambrook et al., 1989). I am familiar with standard methods, and with the Sambrook et al publication that discloses these methods. Such standard methods do not include the preparation of full-length cDNAs nor the use of full-length genes as hybridization partners.

7. It is also important to note that Wagner et al employ a "tiling" methodology in which several hybridization partner fragments overlapping with each other are fixed on a support in order to correspond (as a group) to the complete sequence of a full-length gene. In that method, the availability of short fragments as partners makes it possible to define the position of a mutated base, by observing which "tile" binds the mutated position. The "tiling" methodology therefore requires a high number of fragments (a high number of chips are needed in case of investigation of an entire genomic library) and a high number of mismatch-binding base proteins. In contrast, the method of the present invention allows the detection of a mismatched base in a target sample by using only one full-length DNA as a hybridization partner. This method, which is an "ON-OFF" method, can be used to

immediately detect the presence or absence of a mutation and thereby allow diagnosis of a disease. In my opinion, the use of a full-length gene as hybridization partner is fundamentally incompatible with the tiling methodology carried out by Wagner. The presence of a full-length gene as hybridization partner is thus completely inconsistent with the use of fragments as hybridization partners. The "tiling methodology" and the "full-length" partner methodology are based on a different system, have different applications, and give different results.

8. I find no suggestion in the Wagner et al. PCT publication that would lead me to modify the method used in that publication by employing a full-length gene as a hybridization partner. Moreover, I know of no such suggestion outside of the disclosure of the above-cited application.

9. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

July 23, 2001  
Date

  
Okazaki Yasushi, Ph.D.

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Okazaki Yasushi, Ph.D.